REMARKS

Claims 1-51 remain under active prosecution in the present application. Claims 38-51 are withdrawn from consideration. Applicants respectfully assert that all amendments are supported by the original disclosure and do not introduce new matter. Moreover, Applicants further respectfully assert that the amendments merely clarify the scope of the claims.

Priority

Applicants have amended the first sentence of the specification to correctly claim priority to provisional application 60/139,423, filed 6/16/1999.

Applicants petition the Commissioner to accept an unintentionally delayed claim for priority.

Specification

A new abstract of the disclosure has been presented on a separate sheet, apart from any other text. Applicants direct that the substitute Abstract attached be inserted into the present specification in place of the original. The replacement Abstract does not contain any new matter.

Rejections Under 35 U.S.C. 112, First Paragraph

The Examiner has rejected claims 1-37, under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. Specifically, the Examiner contends the specification does not provide a reasonable limitation for the phrase in claim 1 reciting the limitation "removing substantially all of the chromosomal nucleic acids". The Examiner contends the specification only defines "substantially purified" regarding removal of proteins, salts, lipids, *etc.* (page 7) thus, Examiner contends the removal of chromosomal nucleic acids, the metes and bounds of the claimed subject matter are unclear. Applicants respectfully traverse this rejection.

The application specification describes "substantially purified" as the recovery of nucleic acid which is at least 80% purified with respect to removal of a contaminant, *e.g.*, cellular components such as protein, lipid or salt; thus, the term "substantially purified" generally refers to separation of a majority of cellular proteins or reaction contaminants from the sample, so that compounds capable of interfering with the subsequent use of the isolated nucleic acid are removed. When "removing substantially all of the chromosomal nucleic acids" is read in light of

the "substantially purified" language in the specification, one of ordinary skill in the art is reasonably apprised of the scope of the invention. Removing substantially all of the chromosomal nucleic acids entails removing substantially all of the compounds capable of interfering with the subsequent use of the isolated nucleic acid, extrachromosomal DNA. The isolated extrachromosomal DNA is 80-95% free of contaminating chromosomal DNA and other contaminants.

Claim 1, when read in light of the specification, particularly points out and distinctly claims the invention.

Therefore, the 35 U.S.C. 112, second paragraph, rejection should be withdrawn and claims 1-37 allowed. Reconsideration is respectfully requested.

Rejections Under 35 U.S.C. 102

The Examiner has rejected claim 1 under 35 U.S.C. §102(b) as being anticipated by Little (U.S. patent 5,075,430,1991). The Examiner contends that Little discloses the instantly claimed invention directed to a method of DNA purification comprising alkaline lysis of bacteria followed by neutralization and removal of proteins and chromosomal DNA by centrifugation, immobilization of the supernatant on diatomaceous earth in the presence of a chaotropic agent, and elution of the DNA with water or a low salt buffer.

It is important to note that the present invention discloses materials and methods used in an unexpected sequence to produce high yields of optimally pure extra-chromosomal DNA. Chaotropic agents similar to the ones used in the present invention have been previously used only in the isolation of genomic DNA. Homogenized tissue samples or cell suspensions are treated with chaotropic agents, such as those disclosed in the published application W097/05248, to lyse the cell membranes and release genomic DNA into the lysate. If such materials are used to harvest extra-chromosomal DNA, the resultant release of genomic DNA contaminates the extrachromosomal DNA. In fact, others have attempted to use such materials for isolating plasmid DNA, but have failed to devise the needed modifications to the materials and methods. Therefore, there are no prior art references teaching the use of chaotropic agents for plasmid or other extrachromosomal DNA preparations.

Claim 1 has now been amended to clarify that the method utilizes a lysis step and provides for a chaotropic environment in order to allow the nucleic acids to be removed from the contaminating proteins. These features of the methods are aptly described in the specification and as such there is sufficient basis for the amendment to claim 1.

It is also critical to note that the present methods are for obtaining isolated extrachromosomal DNA without the use of any DNA-binding matrices, such as diatomaceous earth. DNA-binding methods and conventional methods of isolation of extrachromosomal DNA, which use toxic chemicals, result in degradation of the isolated DNA or the recovery of low purity and/or quantity of isolated DNA.

Applicants respectfully submit that amended claim 1 provides sufficient distinction over Little to overcome the rejection. Amended claim 1 describes a method of isolation of extrachromosomal DNA emphasizing a particular sequence of steps using chaotropic agents for the purification step without the use of a DNA-binding material, such as diatomaceous earth. The Little reference discloses the use of diatomaceous earth to purify plasmid DNA.

In direct contravention of these explicit teachings of Little, amended claim 1 of the present application recites a sequence of steps capable of effecting the isolation of extrachromosomal DNA without the use of a DNA-binding material such as diatomaceous earth. The Little reference does not disclose how to achieve the isolation of plasmid DNA without the use of diatomaceous earth. Applicants therefore respectfully submit that, in light of the present amendment to claim 1, amended claim 1 is patentable over Little. Accordingly, Applicants respectfully request that the rejection be withdrawn.

The Examiner has rejected claims 1-2 under 35 U.S.C. 102(b) as being anticipated by Padhye *et al.* (U.S. patent 5,658,548, 1997). Padhye *et al.* teach a plasmid purification protocol comprising alkaline lysis of *E. Coli* in a buffer containing RNase A followed by neutralization and centrifugation to remove proteins and chromosomal DNA (column 11, line 36 - column 12, line 7). To the supernatant (containing plasmid DNA) was added a resin comprising guanidine chloride (a chaotropic agent) and glass particles that bind DNA under these conditions. The glass particles were precipitated by filtration and centrifugation, washed, and DNA eluted with TE buffer (column 13, lines 21-65). Claim 1 has now been amended to clarify that the methods of the present invention utilize a unique sequence of steps, including a lysis step, and provides for a

chaotropic environment in order to allow the nucleic acids to be removed from the contaminating proteins. These features of the methods are aptly described in the specification and as such there is sufficient basis for the amendment to claim 1.

Applicants respectfully submit that the amended claim 1 now provides sufficient distinction over the Padhye *et al.* reference to overcome the rejection. As described above, amended claim 1 describes a method of isolation of extrachromosomal DNA emphasizing a particular sequence of steps using chaotropic agents for the purification step thereby allowing the purification of the extracellular nucleic acids without the use of a DNA-binding material, such as a mixture of silica gel and glass particles. The Padhye *et al.* reference discloses the use of a mixture of silica gel and glass particles to purify plasmid DNA.

In direct contravention of these explicit teachings of Padhye *et al.*, amended claim 1 of the present application recites a sequence of steps capable of effecting the isolation of extrachromosomal DNA without the use of a DNA-binding material, such as a mixture of silica gel and glass particles. The Padhe *et al.* reference does not disclose how to achieve the isolation of plasmid DNA without the use of a mixture of silica gel and glass particles. Applicants therefore respectfully submit that, in light of the present amendment to claim 1, amended claim 1 is patentable over Padhye *et al.* Accordingly, Applicants respectfully request that the rejection be withdrawn.

Rejections Under 35 U.S.C. 103

The Examiner has rejected claims 1-10 and 13-15 under 35 U.S.C. 103(a) as being unpatentable over Little (cited above, 1991) in view of Fisher *et al.* (Analytical Biochem., 1991) and Padhye *et al.* (cited above, 1997). The Examiner contends that it would have been obvious to one of ordinary skill in the art at the time of the invention to modify the teachings of Little to include the use of an RNase A enzyme because it was known in the art at the time of filing that this step can remove unwanted RNA, and because Fisher *et al.* teach this removal is desirable for reasons of DNA purity and function in downstream assays.

According to the Examiner, one would have been motivated to do so in order to receive the expected benefit of improving the purification method taught by Little by removing unwanted RNA. Amended claim 1 describes a method of isolation of extrachromosomal DNA emphasizing

a particular sequence of steps using chaotropic agents for the purification step without the use of a DNA-binding material. The deficiencies of the Little reference have been discussed above. Specifically, Little does not disclose the isolation of extrachromosomal DNA using a particular sequence of steps including chaotropic agents for the purification step without the use of a DNA-binding material as now recited in pending independent claim 1. Padhye *et al.* and Fisher *et al.* are relied upon for disclosing the addition of RNase A to what was described in Little for the removal of unwanted RNA. These references, alone or in combination, do not cure the deficiencies of Little.

Therefore, even if the applied prior art references of record were combined as asserted in the present Office Action, that combination would still fail to disclose or suggest each and every element recited in pending amended independent claim 1. Accordingly, Applicants respectfully request that the rejection be withdrawn.

CONCLUSION

In light of the amendments and remarks made herein, it is respectfully submitted that the claims currently pending in the present application are in form for allowance. Accordingly, reconsideration of those claims, as amended herein, is earnestly solicited. Applicants encourage the Examiner to contact their representative, Stephen R. Albainy-Jenei at (513) 651-6839 or dfranklin@fbtlaw.com.

The Commissioner for Patents is hereby authorized to charge any deficiency or credit any overpayment of fees to Frost Brown Todd LLC Deposit Account No. 06-2226.

Respectfully submitted,

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